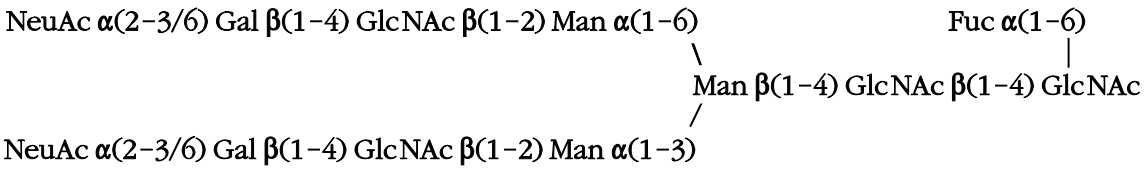
CERTIFICATE OF ANALYSIS

PRODUCT NAME:	GLYKO® DI-SIALYLATED, GALACTOSYLATED, BIANTENNARY COMPLEX N-GLYCAN, CORE SUBSTITUTED WITH FUCOSE (A2F)
PRODUCT CODE:	GKC-224301
LOT NUMBER:	DP08F1001c
PACK SIZE:	10 µg (qualitative standard for glycan identification
PURITY:	87% of glycan by HPLC
FORM:	Dry solid
STORAGE:	Store at -20°C before and after reconstitution
EXPIRATION:	October 2015 may be used for 1 year after reconstitution

STRUCTURE:



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Quality Control:

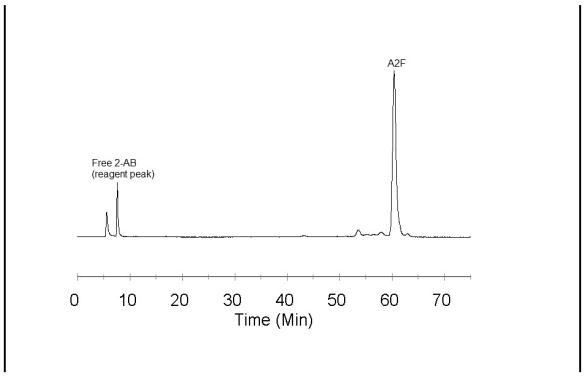


Figure 1 - **HPLC results**:
A2F labeled according to the Signal™ 2-AB Labeling Kit (GKK-404) and analyzed on a GlycoSep™ N column (GKI-4728) in ammonium formate/acetonitrile.

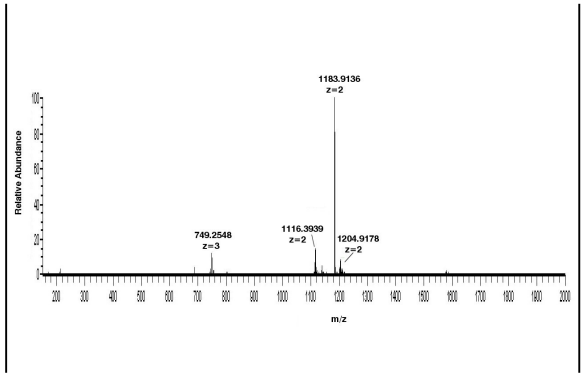


Figure 2 - ESI-MS of A2F $[M - 2H]^{2-}$

Molecular Weight: 2370.2 (average)[1]

Isolation: A2F complex-type N-linked oligosaccharide is typically released from a glycoprotein using N-Glycanase® or anhydrous hydrazine[8], separated from peptide material by adsorption chromatography, then purified further using a combination of glycosidase digestion and chromatographic techniques.

Structural Analysis: The purity and structural integrity of the glycan is assessed by one or more of the following techniques: HPLC[2], mass spectrometry[3,4], FACE[5], ^{1}H-NMR[6] and HPAEC-PAD[7].

Applications:

- qualitative standard for various analytical procedures
- radio-labeling, fluorescent-labeling or formation of a variety of oligosaccharide derivatives
- substrate for glycosidase and glycosyl transferase assays

Reconstitution: Use HPLC-grade water or an aqueous buffer to dissolve the glycan. Store the reconstituted glycan at -20°C in working aliquots. Avoid multiple freeze/thaw cycles.

Handling: The oligosaccharide is shipped as a dried solid. Allow the unopened vial to reach ambient temperature and tap on a solid surface to ensure that most of the material is at the bottom of the vial. Gently remove the cap, add the desired volume of water or buffer, re-cap and mix thoroughly to redissolve all the oligosaccharide. For maximal recovery, ensure that the cap lining is also rinsed, and centrifuge the reconstituted vial briefly before use.

Make sure that any glassware, plasticware, solvents or reagents which come into contact with the glycan are free of glycosidases and carbohydrate contaminants.

Minimize exposure to elevated temperatures or extremes of pH; high temperatures or low pH will cause desialylation. High pH will cause epimerization of the reducing terminal GlcNAc.

REFERENCES

1. Average molecular weight was calculated using the ExPASy GlycanMass calculator: http://us.expasy.org/tools/glycomod/glycanmass.html

2. Guile GR, Rudd PM, Wing DR, Prime SB and Dwek RA. A rapid and high-resolution high-performance liquid chromatographic method for separating glycan mixtures and analyzing oligosaccharide profiles**.** Anal Biochem 1996 Sep 5;240(2):210-226.

3. James DC and Jenkins N. Analysis of N-glycans by matrix-assisted laser desorption/ionization mass spectrometry. In: Jackson P, Gallagher JT, editors. A laboratory guide to glycoconjugate analysis, BioMethods Vol. 9. Basel: Birkhäuser; 1997. p. 91-112.

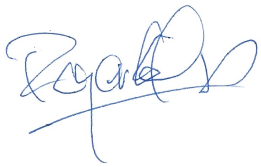
4. Papac DI, Wong A and Jones AJS. Analysis of acidic oligosaccharides by matrix-assisted laser desorption/ionization time of flight mass spectrometry. Anal Chem 1996 Sep 15;68(18):3215-3223.

5. Starr CM, Masada RI, Hague C, Skop E and Klock JC. Fluorophore-assisted carbohydrate electrophoresis in the separation, analysis, and sequencing of carbohydrates. J Chromatogr A 1996 Jan 12;720(1-2):295-321.

6. Vliegenthart JFG, Dorland L and van Halbeek H. High-resolution, ^{1}H-nuclear magnetic resonance spectroscopy as a tool in the structural analysis of carbohydrates related to glycoproteins. Adv Carb Chem Biochem 1983 41: 209-374.

7. Townsend RR, Hardy MR, Hindsgaul O and Lee YC. High-performance anion-exchange chromatography of oligosaccharides using pellicular resins and pulsed amperometric detection. Anal Biochem 1988 Nov 1;174(2):459-70.

8. Takasaki S, Mizouchi T and Kobata A. Hydrazinolysis of asparagine-linked sugar chains to produce free oligosaccharides. Meth Enzymol 1982; 83:263-8.



Roger C Alsop
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Authorized Signature